

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Photochemical production of organic matter triplet states in water samples from mountain lakes, located below or above the tree line**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/120755> since 2016-10-06T10:47:57Z

*Published version:*

DOI:10.1016/j.chemosphere.2012.03.071

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



## UNIVERSITÀ DEGLI STUDI DI TORINO

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in

E. De Laurentiis, M. Minella, V. Maurino, C. Minero, M. Brigante, G. Mailhot, D. Vione. Photochemical Production of Organic Matter Triplet States in Water Samples from Mountain Lakes, Located Below or Above the Treeline. *Chemosphere* **2012**, 88, 1208-1213.

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format:

E. De Laurentiis, M. Minella, V. Maurino, C. Minero, M. Brigante, G. Mailhot, D. Vione. Photochemical Production of Organic Matter Triplet States in Water Samples from Mountain Lakes, Located Below or Above the Treeline. *Chemosphere* **2012**, 88, 1208-1213. DOI: 10.1016/j.chemosphere.2012.03.071 (<http://www.elsevier.com/locate/chemosphere>)

# PHOTOCHEMICAL PRODUCTION OF ORGANIC MATTER TRIPLET STATES IN WATER SAMPLES FROM MOUNTAIN LAKES, LOCATED BELOW OR ABOVE THE TREE LINE

Elisa De Laurentiis,<sup>a</sup> Marco Minella,<sup>a</sup> Valter Maurino,<sup>a</sup> Claudio Minero,<sup>a</sup> Marcello Brigante,<sup>b,c,\*</sup> Gilles Mailhot,<sup>b,c</sup> Davide Vione<sup>a,d,\*</sup>

<sup>a</sup> *Dipartimento di Chimica, Università di Torino, Via P. Giuria 5, 10125 Torino, Italy.*  
*<http://www.environmentalchemistry.unito.it>*

<sup>b</sup> *Clermont Université, Université Blaise Pascal, Institut de Chimie de Clermont-Ferrand (ICCF)-ENSCCF, BP 10448, F-63000 Clermont-Ferrand.*

<sup>c</sup> *CNRS, UMR 6296, Institut de Chimie de Clermont-Ferrand, F-63171 Aubière*

<sup>d</sup> *Centro Interdipartimentale NatRisk, Università di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy.* *<http://www.natrisk.org>*

\* Address correspondence to either author. E-mail: [marcello.brigante@univ-bpclermont.fr](mailto:marcello.brigante@univ-bpclermont.fr) (M.B.); [davide.vione@unito.it](mailto:davide.vione@unito.it) (D.V.).

## Abstract

The production of triplet states (T\*) of chromophoric dissolved organic matter (CDOM), reacting with the probe molecule 2,4,6-trimethylphenol (TMP) was measured upon irradiation of water samples, taken from lakes located in a mountain area (NW Italy) between 1450 and 2750 m above sea level. The lakes are located below or above the tree line and surrounded by different vegetation types (trees, alpine meadows or exposed rocks). The most photoactive samples belonged to lakes below the tree line and their fluorescence spectra and CDOM optical features suggested the presence of a relatively elevated amount of humic (allochthonous) material. The lowest (negligible) photoactivity was found for a lake surrounded by exposed rocks. Its CDOM showed an important autochthonous contribution (due to in-lake productivity) and considerably higher spectral slope compared to the other samples, suggesting low CDOM molecular weight and/or aromaticity. Among the samples, CDOM photoactivity (measured as the rate of TMP-reactive T\* photoproduction) decreased with changing vegetation type in the order: trees, meadows, rocks. It

could be connected with decreasing contribution from catchment runoff and increasing contribution from autochthonous processes and possibly precipitation.

*Keywords:* lake water photochemistry; sensitised photolysis; inter-system crossing; 2,4,6-trimethylphenol; humic and fulvic acids; fluorescence matrix (EEM).

## *Introduction*

Mountain lakes are peculiar environments that are highly impacted by solar radiation. A combination of altitude and low concentration of UV-absorbing substances favours the penetration of harmful UV radiation in the water column (Laurion et al., 2000). Despite the development of several adaptations by aquatic organisms (Sommaruga, 2010), alpine lakes are a potentially vulnerable environment to climate change because of the possible increase of UV doses connected to variations in organic matter inputs and, most notably, to decreases in ice cover (Neale et al., 2001; Leavitt et al., 2003; Rogora et al., 2003).

Chromophoric dissolved organic matter (CDOM) is the main radiation absorber in lake water in the 300-500 nm wavelength range (Bracchini et al., 2004). DOM/CDOM can be produced by aquatic organisms (autochthonous) or reach the lake by catchment runoff (allochthonous) (Gondar et al., 2008). In alpine environments, catchment runoff is expected to be a more important DOM source for lakes located below the tree line (Vinebrooke and Leavitt, 1998). A recent study has also shown that high-altitude alpine lakes are comparatively more impacted by atmospheric deposition, and that a significant fraction of organic matter including pollutant load would be of atmospheric origin (Mladenov et al., 2011).

An important feature of CDOM is its photoactivity, namely the ability to produce reactive species upon sunlight absorption. A key issue is the photoproduction of triplet states ( $T^*$ ), which can be reactive themselves and/or yield  $^1O_2$  and  $\bullet OH$  (Canonica, 2007; Latch and McNeill, 2006). These reactive transients can induce transformation of dissolved pollutants or of naturally occurring DOM (Richard et al., 2007), and might also cause oxidative stress to living organisms (Souza et al., 2007). Such considerations account for the importance of measuring reactive species photoproduction upon irradiation of water from mountain lakes, and to our knowledge information on this subject is very scarce.

We have recently observed negligible CDOM photoactivity in rainwater (Albinet et al., 2010b). Considering that lakes located in different mountain environments across the tree line (dominated

by forests, meadows or rocks) would be impacted to a different extent by catchment runoff, in-water productivity and atmospheric deposition, one might expect significant differences in CDOM photoactivity. However, to our knowledge such a hypothesis has never been tested. In this work, we measured the photochemical generation of TMP-reactive T\* in water samples from lakes located below and above the tree line, surrounded by trees, meadows or exposed rocks. An attempt was made to relate CDOM photoactivity with water chemical composition and environmental features.

## *Experimental*

**Reagents and materials.** H<sub>3</sub>PO<sub>4</sub> (85%), NaNO<sub>2</sub> (>97.5%), 2,4-dinitrophenylhydrazine (98%) and 2,4,6-trimethylphenol (TMP, 99%) were purchased from Aldrich, NaNO<sub>3</sub> (>99.5%), NaHCO<sub>3</sub> (99%), Na<sub>2</sub>CO<sub>3</sub> (99%), CCl<sub>4</sub> (Uvasol for spectroscopy) and methanol (LiChrosolv gradient grade) from VWR Int., Zero-grade air from SIAD (Bergamo, Italy). Water used was of Milli-Q quality.

**Sampling and sample treatment.** The study lakes are located in Torino province, NW Italy. Water samples were taken from the surface layer of the lakes under study with a glass bottle secured to a 10-m rope, to avoid entering the lake and perturbing the sediment. Samples were transported to the laboratory under refrigeration. Sampling dates and lake features are reported in Table 1. The samples were vacuum-filtered on Millipore MF membranes (cellulose acetate, pore diameter 0.45 µm) and stored under refrigeration till further processing.

**Determination of nitrate, nitrite, DOC and pH.** Nitrate was determined by ion chromatography, nitrite by high performance liquid chromatography (HPLC) upon pre-column derivatisation with 2,4-dinitrophenylhydrazine (Kieber and Seaton, 1996). DOC was measured as Non-Purgeable Organic Carbon (NPOC) with a Shimadzu TOC-V<sub>CSH</sub> Total Organic Carbon Analyzer. Sample pH was measured with a Metrohm 602 combined glass electrode, connected to a Metrohm 713 pH meter. Further details are reported as Supplementary Material (hereafter SM).

**Fluorescence measurements.** A Varian Cary Eclipse fluorescence spectrofluorimeter was used, adopting a 10 nm bandpass on both excitation and emission. Fluorescence excitation-emission matrix (EMM) was obtained at 5 nm intervals for excitation wavelengths from 250 to 460 nm and emission ones from 250 to 600 nm. Identification of fluorescent constituents in water samples was performed on the basis of literature data, using the main contours to identify fluorescence peaks.

**Irradiation experiments.** Lake water samples (20 mL) were spiked with 10  $\mu\text{M}$  TMP and placed into cylindrical Pyrex glass cells (diameter 4.0 cm, height 2.5 cm), tightly closed with a lateral screw cap. The cells were irradiated from the top under a set of five UVA lamps (TL K 40W/05, Philips, emission maximum 365 nm), with magnetic stirring. Two replicates were made of each irradiation experiment and the results were averaged. Blank runs were carried out, in the absence of irradiation, by wrapping the cells with aluminium foil and placing them under the lamp. The time evolution of TMP was also monitored upon irradiation in Milli-Q water.

The UV irradiance (295-400 nm) reaching the cells was  $22 \pm 1 \text{ W m}^{-2}$ , measured with a CO.FO.ME.GRA. (Milan, Italy) power meter. The photon flux in solution was  $1.6 \times 10^{-5} \text{ Einstein L}^{-1} \text{ s}^{-1}$ , actinometrically determined with the ferrioxalate method (Kuhn et al., 2004). Figure 1 shows the emission spectrum of the lamps, taken with an Ocean Optics SD2000 CCD spectrophotometer and normalised to the actinometry data, taking into account the Pyrex transmittance (Albinet et al., 2010a). The Figure also reports the absorption spectra of the filtered lake water samples ( $A_1(\lambda) = A(\lambda) b^{-1}$ , where  $A(\lambda)$  is the measured absorbance and  $b$  the optical path length), taken on a Varian CARY 300 UV-Vis spectrophotometer with a cylindrical quartz cuvette having  $b = 10 \text{ cm}$ .

Irradiation time was up to 32 h, which approximately corresponds to the sunlight UV energy input of three fair-weather summer days at  $45^\circ\text{N}$  latitude (Maddigapu et al., 2011). TMP after irradiation was monitored by HPLC. The isocratic eluent was a 60:40 mixture of methanol: aqueous  $\text{H}_3\text{PO}_4$  (pH 2.8) at a flow rate of  $1.0 \text{ mL min}^{-1}$ . The TMP retention time was 6.7 min, detection wavelength 210 nm.

**Assessment of the rates of triplet state formation.** Radiation absorption by organic compounds in surface water yields the excited singlet states, which by inter-system crossing can be transformed into the longer-lived triplet states,  $\text{T}^*$  (Hoigné, 1990).  $\text{T}^*$  would mainly react with  $\text{O}_2$  to give  $^1\text{O}_2$  or undergo various deactivation reactions. Such processes have a lumped rate constant  $k' \approx 5 \cdot 10^5 \text{ s}^{-1}$  (Canonica and Freiburghaus, 2001). Moreover,  $\text{T}^*$  can also react with dissolved organic molecules.

TMP is a suitable probe to measure the formation rate of  $\text{T}^*$ ,  $R_{\text{T}^*}$  (Halladja et al., 2007; Minella et al., 2011) due to its selectivity. TMP can also react with  $^1\text{O}_2$ , but the  $^1\text{O}_2$  contribution to TMP transformation would be negligible compared to  $\text{T}^*$  (see SM).

Assume  $R_{\text{TMP}}$  as the experimentally measured initial rate of TMP transformation in lake water, while  $R_0$  is the rate found upon irradiation of TMP in Milli-Q water, due to different unaccounted for processes (e.g. direct photolysis, volatilisation). No TMP transformation was detected with lake

water in the dark, excluding important microbiological processes. Therefore, the difference  $R_{\text{TMP}} - R_o$  would represent the TMP transformation rate that is accounted for by  $T^*$ . Considering the competition for  $T^*$  between TMP (with second-order rate constant  $k_{\text{TMP},T^*} = 3.0 \cdot 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ; Al Housari et al., 2010) and the other deactivation processes (with pseudo-first order rate constant  $k' = 5 \cdot 10^5 \text{ s}^{-1}$ , Canonica and Freiburghaus, 2001) and applying the steady-state approximation to  $T^*$ , the initial rate of TMP-reactive triplet formation  $R_{T^*}$  can be obtained as follows (the complete kinetic treatment is reported as SM):

$$R_{T^*} = (R_{\text{TMP}} - R_o) \cdot \frac{k_{\text{TMP},T^*} [\text{TMP}]_o + k'}{k_{\text{TMP},T^*} [\text{TMP}]_o} \quad (1)$$

where  $[\text{TMP}]_o = 10 \text{ }\mu\text{M}$  is the initial TMP concentration. The TMP time trends were fitted with equations  $[\text{TMP}]_t = [\text{TMP}]_o (1 - k t)$ , obtaining  $R_{\text{TMP}} = k [\text{TMP}]_o$ . The linear time trend of TMP suggests zero-order kinetics, probably due to TMP being a major  $T^*$  sink under the adopted conditions (Minella et al., 2011).

**Numerical data treatment.** Linear and non-linear fits (to calculate reaction rates and spectral slopes, respectively) were carried out with the FigP software package (BioSoft, UK).

## *Results and discussion*

**$T^*$  formation rate.** Figure 2 reports the time trend of TMP, added to the lake water samples at  $10 \text{ }\mu\text{M}$  initial concentration, upon UVA irradiation. The corresponding values of  $R_{\text{TMP}}$  and  $R_{T^*}$  are reported in Table 1. Two samples (from Lago Nero and Lago della Foppa, which are both located below the tree line) had markedly higher  $R_{T^*}$  values than the others. On the other hand, negligible  $R_{T^*}$  was observed in the case of Lago Rouen, which is mostly surrounded by exposed rocks. Such an environment is expected to decrease the importance of soil runoff as organic matter source (Laurion et al., 2000). Soil-derived organic matter usually includes a considerable fraction of humic substances (Vinebrooke and Leavitt, 1998) with significant photochemical activity (Halladja et al., 2007). Moreover, precipitation might be a significant contributor to lake water chemical composition above the tree line and with no meadows around the lake (Mladenov et al., 2011), and there is some evidence for rainwater CDOM to be less photoactive than that of surface water (Albinet et al., 2010b).

The polychromatic quantum yield of T\* formation was assessed for the different lake water samples, as  $\Phi_{T^*} = R_{T^*} P_a^{-1}$ , where  $P_a$  is the photon flux absorbed by lake water. From the water absorption coefficient  $A_1(\lambda)$  and the incident spectral photon flux density of the lamp,  $p^\circ(\lambda)$  (Figure 1), one derives the absorbed photon flux:

$$P_a = \int_{\lambda} p^\circ(\lambda) \cdot [1 - 10^{-A_1(\lambda) \cdot b}] d\lambda \quad (2)$$

where  $b = 1.6$  cm is the optical path length of the irradiated solutions. The lamp and lake water spectra overlap in the 300-500 nm wavelength range, where CDOM is expected to be the main radiation absorber (Bracchini et al., 2004).  $\Phi_{T^*}$  values for the different samples are reported in Table 1, and Lago Verde and Lago della Foppa (both located below the tree line) showed the highest  $\Phi_{T^*}$ .

**Spectrophotometric characterisation of lake water CDOM.** CDOM was characterised for DOC-specific absorbance (Rostan and Cellot, 1995; Westerhoff and Anning, 2000) and spectral slope (Vodacek et al., 1997). As DOC-specific absorbance we adopted the ratio  $A_1(254\text{nm}) \text{ DOC}^{-1}$  between the absorption coefficient at 254 nm ( $\text{cm}^{-1}$ ) and the DOC value ( $\text{g C L}^{-1}$ ) (Oliveira et al., 2006). The spectral slope  $S$  was determined by fitting the lake water  $A_1(\lambda)$  data between 295 and 400 nm with the exponential equation  $A_1(\lambda) = A_0 e^{-S \lambda}$  (Laurion et al., 2000), with  $A_0$  and  $S$  as fit parameters. Most of the lake water  $A_1(\lambda)$  spectra showed good exponential decays with wavelength (see Figure 1). The spectrum of Lago Rouen had a small peak between 300 and 335 nm (Figure 1), which is usually associated to material released from plankton (Vernet and Whitehead, 1996). The wavelengths associated to that peak were not considered in the exponential fit (Laurion et al., 2000), but this procedure had limited effect on  $S$ : peak elimination decreased the measured  $S$  value of Lago Rouen by less than 10%. A further spectrophotometric variable considered is the spectral index  $E_2/E_3$  (ratio of the absorbance values at 250 and 365 nm).

The values of  $A_1(254\text{nm}) \text{ DOC}^{-1}$ ,  $E_2/E_3$  and  $S$  for the samples are reported in Table 1. Note the elevated  $A_1(254\text{nm}) \text{ DOC}^{-1}$  of Lago Nero ( $> 20 \text{ cm L (g C)}^{-1}$ ), which might be associated to the presence of abundant Humic-like substances (possibly fulvic acids). In contrast, values lower than 10 observed for Lago Verde, Rosset and Rouen suggest significant occurrence of non-absorbing aliphatic material (Oliveira et al., 2006).



The  $S$  values are in the  $12\text{--}19\ \mu\text{m}^{-1}$  range, which is typical of mountain lakes (Laurion et al., 2000). Usually,  $S$  is negatively correlated to the molecular weight and/or aromaticity degree of CDOM, as is the index  $E_2/E_3$  (Table 1) (Peuravuori and Pihlaja, 1997).

Interestingly, the Lago Rouen sample had the highest  $S$  and  $E_2/E_3$  values, which is usually linked to low occurrence of humic substances (Oliveira et al., 2006). The latter mainly reach lake water from catchment runoff (Vinebrooke and Leavitt, 1998), but would be leached in limited amount from an environment dominated by exposed rocks (Laurion et al., 2000). Contribution of precipitation could also be important in such an environment (Mladenov et al., 2011).

It is interesting to compare the  $S$  values reported in Table 1 with those derived by exponential fit of rainwater spectra, collected in the same region of the lakes (Albinet et al., 2010b). The much scattered  $S$  values of rainwater range from 12 to over  $100\ \mu\text{m}^{-1}$ , but they are on average considerably higher compared to lake water. This finding is reasonable, considering that organic compounds in the atmosphere have usually lower molecular weight than those in surface waters (Graber and Rudich, 2006). In fact, while photochemical reactions in the atmosphere could lead to formation of oligomers with molecular weight up to 900 Da (Grgic et al., 2010), humic substances in surface waters are over ten times larger (Retamal et al., 2007).

**Fluorescence characterisation of lake water CDOM.** Fluorescent constituents were identified on the basis of previously reported data (Coble, 1996; Baker, 2001). Figure 3 reports the EMM plots of Lago Nero, della Foppa, Nivolet and Rouen. Several types of fluorescence signals are observed that can be attributed to Tryptophan-like (autochthonous) and Humic-like (allochthonous) material. The other EEM plots (Lago Verde, Soprano, Sottano, Rosset) are reported in Figure 2-SM. The signals are characterised by  $Ex_{\text{max}}$  and  $Em_{\text{max}}$ , the respective wavelengths of maximum excitation and emission. The linear features are the first and second harmonic of the Rayleigh-Tyndall scatter (emission wavelength equal to or double than excitation wavelength), and the Raman scattering of water (Baker, 2002).

For Lago Nero we observed two main signals corresponding to an intense Humic-like peak A ( $Ex_{\text{max}}/Em_{\text{max}}$  250-275 nm/400-480 nm) and to Humic-like peak C ( $Ex_{\text{max}}/Em_{\text{max}}$  305-330 nm/410-450 nm). Lago Verde showed a major peak corresponding to Tryptophan-like compounds (peak T,  $Ex_{\text{max}}/Em_{\text{max}}$  270-280 nm/330-360 nm) and, with lower intensity, Humic-like peak A at  $Ex_{\text{max}}/Em_{\text{max}}$  around 250/435 nm. In Lago Nivolet and Lago Rosset only the Tryptophan-like signal was observed. A similar signal was present in Lago della Foppa, together with a smaller fluorescence peak at  $Ex_{\text{max}}/Em_{\text{max}}$  250-254 nm/420-460 nm that can be attributed to Humic-like material (“peak A”; Coble, 1996).

Lago Soprano and Sottano, with similar EMM, have contours corresponding to Tryptophan-like compounds, peak A of Humic-like material (with very low intensity for Lago Sottano) and a further peak located at  $Ex_{max}/Em_{max} = 310\text{-}320\text{ nm}/370\text{-}400\text{ nm}$ , which was also found in Lago Rouen as its only important EEM feature. Such a peak (PD) has been described previously and assigned to material arising from phytoplankton decomposition (Zhang et al., 2009). It is most likely a signature of autochthonous organic matter.

All lake samples above the tree line have autochthonous organic material, which is consistent with an important role of in-lake productivity compared to catchment runoff. In contrast, Humic-like material was detected in all samples from lakes below the tree line.

**Principal Component Analysis (PCA) of the data.** After column autoscaling, the data matrix reported in Table 1 (underlined variables) was subjected to PCA to get insight into possible correlations. PCA was carried out with the free chemometric software V-Parvus 2008 (Forina et al., 2008). The first two principal components explained 67% of total variance, and PC3 explained a further 16%. Figure 4 reports the plot of PC1 vs. PC2.  $R_T^*$  and  $\Phi_T^*$ , as well as inorganic carbon, showed good correlation with high positive loadings on PC<sub>1</sub> and low loadings on PC<sub>2</sub>. PC<sub>1</sub> could thus be able to differentiate the photochemical activity of the samples. It is also interesting to see an anticorrelation (loadings pointing in opposite directions) between  $\Phi_T^*$  and the variables  $S$  and  $E_2/E_3$ , which suggests that  $\Phi_T^*$  would be higher in samples where CDOM has higher aromaticity/molecular weight (low  $S$  and  $E_2/E_3$ ). This finding is consistent with the significant photochemical activity of humic substances (Halladja et al., 2007).

The plot of PC1 vs. PC2 does not show a clear differentiation between the lake water samples. However, the sample scores on PC1 suggest some agreement between photoactivity data and environmental features derived from the kind of vegetation surrounding the lakes. In fact, two of the samples from lakes below the tree line (Lago Nero and Lago della Foppa) have elevated positive scores on PC1. In contrast, Lago Rouen that is surrounded by rocks with practically no vegetation has a highly negative score on PC1, and all the other samples have relatively low absolute values.

It was unfortunately not possible to quantify the vegetation type. However, Spearman's rank correlation for ordinal data was applied with the following vegetation ranking: trees – meadows – rocks. The test gave statistically significant correlation between  $R_T^*$  and vegetation type ( $p = 0.039$ ). By comparison, insignificant rank correlation with vegetation type was found for DOC, nitrate, nitrite or for the spectral features  $A_1(254\text{nm})\text{ DOC}^{-1}$ ,  $S$  and  $E_2/E_3$ .

## *Conclusions*

In the present study, the highest TMP-reactive T\* photoproduction was observed in samples from lakes located below the tree line. The CDOM in the most photoactive samples had relatively high values of  $A_1(254\text{nm}) \text{ DOC}^{-1}$  (Table 1) and significant levels of humic substances as shown by EEM (Figure 3). Samples from lakes above the tree line and surrounded by alpine meadows had usually lower values of T\* photoproduction. Their CDOM spectral features were very variable, but EEM data suggested an important contribution from autochthonous CDOM sources. Water from Lago Rouen, located above the tree line and surrounded by rocks with no meadows showed negligible photoactivity and low  $A_1(254\text{nm}) \text{ DOC}^{-1}$ . Its CDOM also had the highest values of spectral slope  $S$  and  $E_2/E_3$  index, suggesting low molecular weight and aromaticity. This implies low CDOM contribution from catchment runoff, while EEM suggested the presence of autochthonous material.

Overall, the generation rate of TMP-reactive T\* showed better rank correlation than chemical composition with the vegetation type surrounding the lakes. This finding suggests that, at least in the studied samples, photochemistry could be more sensitive than water chemistry to the lake location with respect to the tree line. Further studies will be needed to check the validity of this assumption.

## *Acknowledgements*

The PhD grant of EDL was financially supported by Progetto Lagrange – Fondazione CRT, Torino, Italy. MB, GM and DV acknowledge financial support from EGIDE and Università Italo-Francese – Progetto Galileo.

## *References*

- Al Housari, F., Vione, D., Chiron, S., Barbati, S., 2010. Reactive photoinduced species in estuarine waters. Characterization of hydroxyl radical, singlet oxygen and dissolved organic matter triplet state in natural oxidation processes. *Photochem. Photobiol. Sci.* 9, 78-86.
- Albinet, A., Minero, C., Vione, D., 2010a. Phototransformation processes of 2,4-dinitrophenol, relevant to atmospheric water droplets. *Chemosphere* 80, 753-758.

- Albinet, A., Minero, C., Vione, D., 2010b. Photochemical generation of reactive species upon irradiation of rainwater: Negligible photoactivity of dissolved organic matter. *Sci. Total Environ.* 408, 3367-3373.
- Baker A., 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. *Environ. Sci. Technol.* 35, 948-953.
- Baker, A., 2002. Fluorescence excitation-emission matrix characterization of river waters impacted by a tissue mill effluent. *Environ. Sci. Technol.* 36, 1377-1382.
- Bracchini, L., Loisel, S., Dattilo, A. M., Mazzuoli, S., Cozar, A., Rossi, C., 2004. The spatial distribution of optical properties in the ultraviolet and visible in an aquatic ecosystem. *Photochem. Photobiol.* 80, 139-149.
- Canonica, S., Freiburghaus, M., 2001. Electron-rich phenols for probing the photochemical reactivity of freshwaters. *Environ. Sci. Technol.* 35, 690-695.
- Canonica, S., 2007. Oxidation of aquatic organic contaminants induced by excited triplet states. *Chimia* 61, 641-644.
- Coble, P. G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission spectroscopy. *Mar. Chem.* 51, 325-346.
- Forina, M., Lanteri, S., Armanino, C., Casolino, C., Casale, M., Olivieri, P. 2008. V-Parvus 2008. <http://www.parvus.unige.it>, last accessed November 2011.
- Gondar, D., Thacker, S. A., Tipping, E., Baker, A., 2008. Functional variability of dissolved organic matter from the surface water of a productive lake. *Wat. Res.* 42, 81-90.
- Graber, E. R., Rudich, Y., 2006. Atmospheric HULIS: How humic-like are they? A comprehensive and critical review. *Atmos. Chem. Phys.* 6, 729-753.
- Grgić, I., Nieto-Gligorovski, L.I., Net, S., Temime-Roussel, B., Gligorovski, S., Wortham H., 2010. Light induced multiphase chemistry of gas-phase ozone on aqueous pyruvic and oxalic acids. *Phys. Chem. Chem. Phys.* 12, 698-707.
- Halladja, S., Ter Halle, A., Aguer, J. P., Boulkamh, A., Richard, C., 2007. Inhibition of humic substances mediates photooxygenation of furfuryl alcohol by 2,4,6-trimethylphenol. Evidence for reactivity of the phenol with humic triplet excited states. *Environ. Sci. Technol.* 2007, 41, 6066-6073.
- Hoigné, J., 1990. Formulation and calibration of environmental reaction kinetics: Oxidations by aqueous photooxidants as an example. In: *Aquatic Chemical Kinetics*, Stumm, W. (Ed.), Wiley, NY, pp. 43-70.
- Kieber, R. J., Seaton, P. J., 1996. Determination of subnanomolar concentrations of nitrite in natural waters. *Anal. Chem.* 67, 3261-3264.

- Kuhn, H. J., Braslavsky, S. E., Schmidt, R., 2004. Chemical actinometry. *Pure Appl. Chem.* 76, 2105-2146.
- Latch, D. E., McNeill, K., 2006. Microheterogeneity of singlet oxygen distributions in irradiated humic acid solutions. *Science* 311, 1743-1747.
- Laurion, I., Ventura, M., Catalan, J., Psenner, R., Sommaruga, R., 2000. Attenuation of ultraviolet radiation in mountain lakes: Factors controlling the among- and within-lake variability. *Limnol. Oceanogr.* 45, 1274-1288.
- Leavitt, P. R., Cumming, B. F., Smol, J. P., Reasoner, M., Pienitz, R., Hodgson, D., 2003. Climatic control of ultraviolet radiation effects on lakes. *Limnol. Oceanogr.* 48, 2062-2069.
- Maddigapu, P. R., Minella, M., Vione, D., Maurino, V., Minero, C., 2011. Modeling phototransformation reactions in surface water bodies: 2,4-Dichloro-6-nitrophenol as a case study. *Environ. Sci. Technol.* 45, 209-214.
- Minella, M., Romeo, F., Vione, D., Maurino, V., Minero, C., 2011. Low to negligible photoactivity of lake-water matter in the size range from 0.1 to 5  $\mu\text{m}$ . *Chemosphere* 83, 1480-1485.
- Mladenov, N., Sommaruga, R., Morales-Baquero, R., Laurion, I., Camarero, L., Dieguez, M. C., Camacho, A., Delgado, A., Torres, O., Chen, Z., Felip, M., Reche, I., 2011. Dust inputs and bacteria influence dissolved organic matter in clear alpine lakes. *Nat. Commun.* 2, article 405.
- Neale, P. J., Litchman, E., Sobrino, C., Callieri, C., Morabito, G., Montecino, V., Huot, Y., Bossard, P., Lehmann, C., Steiner, D., 2001. Quantifying the response of phytoplankton photosynthesis to ultraviolet radiation: Biological weighting functions versus in situ measurements in two Swiss lakes. *Aquat. Sci.* 63, 265-285.
- Oliveira, J. L., Boroski, M., Azevedo, J. C. R., Nozaki, J., 2006. Spectroscopic investigation of humic substances in a tropical lake during a complete hydrological cycle. *Acta Hydrochim. Hydrobiol.* 34, 608-617.
- Peuravuori, J., Pihlaja, K., 1997. Molecular size distribution and spectroscopic properties of aquatic humic substances. *Anal. Chim. Acta* 337, 133-149.
- Retamal, L., Vincent, W. F., Martineau, C., Osburn, C. L., 2007. Comparison of the optical properties of dissolved organic matter in two river-influenced coastal regions of the Canadian Arctic. *Estuar. Coast. Shelf Sci.* 72, 261-272.
- Richard, C., Ter Halle, A., Brahmia, O., Malouki, M., Halladja, S., 2007. Auto-remediation of surface waters by solar-light: Photolysis of 1-naphthol and two herbicides in pure and synthetic waters. *Catal. Today* 124, 82-87.
- Rogora, M., Mosello, R., Arisci, S., 2003. The effect of climate warming on the hydrochemistry of Alpine Lakes. *Water Air and Soil Pollut.* 148, 347-361

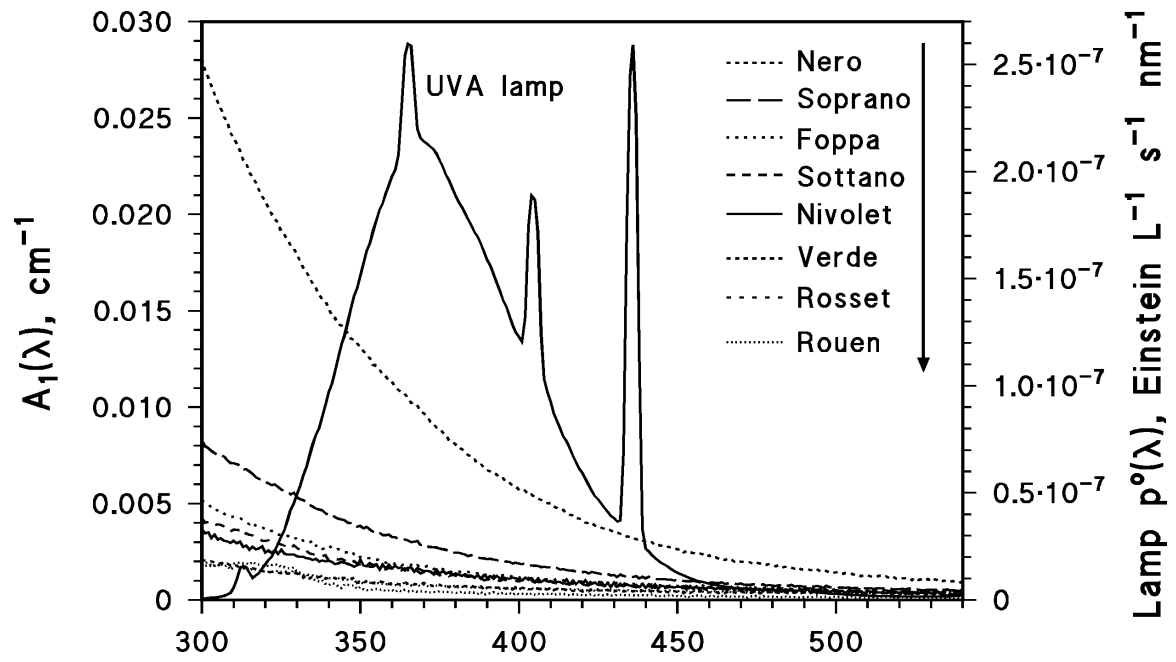
- Rostan, J. C., Cellot, B., 1995. On the use of UV spectrophotometry to assess dissolved organic carbon origin variations in the Upper Rhône River. *Aquat. Sci.* 57, 70-80.
- Sommaruga, R., 2000. Preferential accumulation of carotenoids rather than of mycosporine-like amino acids in copepods from high altitude Himalayan lakes. *Hydrobiologia* 648, 143-156.
- Souza, M. S., Modenutti, B. E., Balseiro, E. G., 2007. Antioxidant defences in planktonic crustaceans exposed to different underwater light irradiances in Andean lakes. *Water Air Soil Pollut.* 183, 49-57.
- Vernet, M., Whitehead, K., 1996. Release of ultraviolet-absorbing compounds by the red-tide dinoflagellate *Lingulodinium polyedra*. *Mar. Biol.* 127, 35-44.
- Vinebrooke, R. D., Leavitt, P. R., 1998. Direct and interactive effects of allochthonous dissolved organic matter, inorganic nutrients, and ultraviolet radiation on an alpine littoral food web. *Limnol. Oceanogr.* 43, 1065-1081.
- Vodacek, A., Blough, N. V., DeGrandpre, M. D., Peltzer, E. T., Nelson, R. K., 1997. Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. *Limnol. Oceanogr.* 42, 674-686.
- Westerhoff, P., Anning, D., 2000. Concentrations and characteristics of organic carbon in surface water in Arizona: influence of urbanization. *J. Hydrol.* 236, 202-222.
- Zhang, Y., van Dijk, M. A., Liu, M., Zhu, G., Qin, B., 2009. The contribution of phytoplankton degradation to chromophoric dissolved organic matter (CDOM) in eutrophic shallow lakes: Field and experimental evidence. *Wat. Res.* 43, 4685-4697.

**Table 1.** Features of the sampled lakes. IC: inorganic carbon ( $\text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^{2-}$ ). Note:  $(1.1 \pm 0.1)\text{E}-10$  is a compact notation for  $(1.1 \pm 0.1) \cdot 10^{-10}$ , and so on. n/a: not applicable. The average depth of the lakes is in the range of 1-2 m.  $E_2/E_3$  is the ratio between the absorbance values at 250 and 365 nm (Peuravuori and Pihlaja, 1997). The error bounds represent  $\mu \pm \sigma$ . The underlined variables were included in PCA.

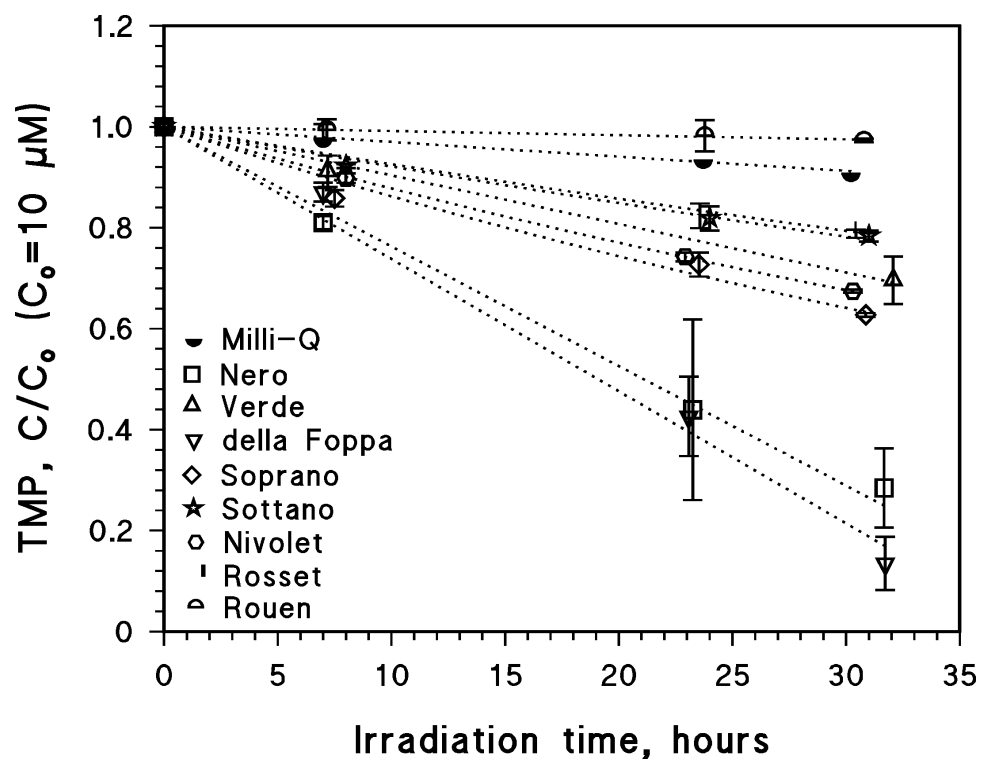
Lake	Altitude, m	Sampling date	Surface, km <sup>2</sup>	Tree line	Surrounding environment	pH	$P_{\text{a}},$ $10^{-7} \text{ Ein L}^{-1} \text{ s}^{-1}$			
<i>Lago Nero</i>	2070	1 Sep 2011	0.110	Below	Trees (fir), water plants, meadows	7.2	5.1			
<i>Lago Verde</i>	1840	1 Sep 2011	0.012	Below	Trees (fir)	7.4	4.5			
<i>Lago della Foppa</i>	1450	1 Sep 2011	0.016	Below	Trees (deciduous)	7.5	0.96			
<i>Lago Nivolet</i>	2750	30 Aug 2011	0.010	Above	Meadows, some rocks	6.7	0.82			
<i>Lago Rosset</i>	2700	30 Aug 2011	0.166	Above	Meadows	7.4	0.44			
<i>Lago Soprano</i>	2100	31 Aug 2011	0.018	Above	Meadows, some rocks	6.8	1.6			
<i>Lago Sottano</i>	2210	31 Aug 2011	0.011	Above	Meadows, some rocks	6.8	0.80			
<i>Lago Rouen</i>	2390	31 Aug 2011	0.018	Above	Rocks, no meadows	7.5	0.28			
	$R_{\text{TMP}},$ $10^{-11} \text{ M s}^{-1}$	$R_{\text{T}*},$ $10^{-9} \text{ M s}^{-1}$	$\Phi_{\text{T}*}, 10^{-3}$	<u>DOC</u> , $\text{mg C L}^{-1}$	<u>IC</u> , $\text{mg C L}^{-1}$	<u>NO<sub>3</sub><sup>-</sup></u> , $\mu\text{M}$	<u>NO<sub>2</sub><sup>-</sup></u> , $\mu\text{M}$	<u>A<sub>1</sub>(254nm)/DOC</u> , $\text{cm L/ gC}$	<u>S</u> , $\mu\text{m}^{-1}$	<u>E<sub>2</sub>/E<sub>3</sub></u>
<i>Nero</i>	11±1	1.8±0.1	3.5±0.2	1.80±0.06	28.4±6.8	< 0.8	0.36	29.8±1.0	15.5±0.1	5.28
<i>Verde</i>	3.2±0.1	0.41±0.03	9.1±0.7	0.43±0.02	19.0±3.0	12.7	0.28	8.6±0.4	13.7±0.2	4.64
<i>Foppa</i>	12±2	1.9±0.4	2.0±0.4	0.53±0.01	23.6±4.4	6.01	< 0.02	19.0±0.4	15.6±0.1	5.80
<i>Nivolet</i>	3.6±0.1	0.49±0.01	6.0±0.2	0.62±0.01	14.9±2.1	1.68	0.04	13.0±0.2	12.1±0.2	5.05
<i>Rosset</i>	2.1±0.2	0.22±0.04	5.0±0.9	0.64±0.01	12.2±1.0	< 0.8	0.31	7.8±0.1	13.5±0.2	6.68
<i>Soprano</i>	4.1±0.2	0.57±0.05	3.6±0.3	0.84±0.04	1.08±0.01	25.4	1.07	18.7±0.9	15.0±0.1	5.37

<i><b>Sottano</b></i>	2.3±0.1	0.25±0.02	3.1±0.2	0.48±0.01	0.16±0.01	28.9	0.24	17.1±0.4	15.5±0.1	5.64
<i><b>Rouen</b></i>	0.23±0.01	Negligible	Negligible	0.53±0.01	0.56±0.01	8.52	0.69	9.1±0.2	18.9±0.4	11.3
<i><b>Milli-Q</b></i>	0.85±0.03	n/a	n/a	0.13±0.01	0	< 0.8	< 0.02	~ 0	n/a	n/a

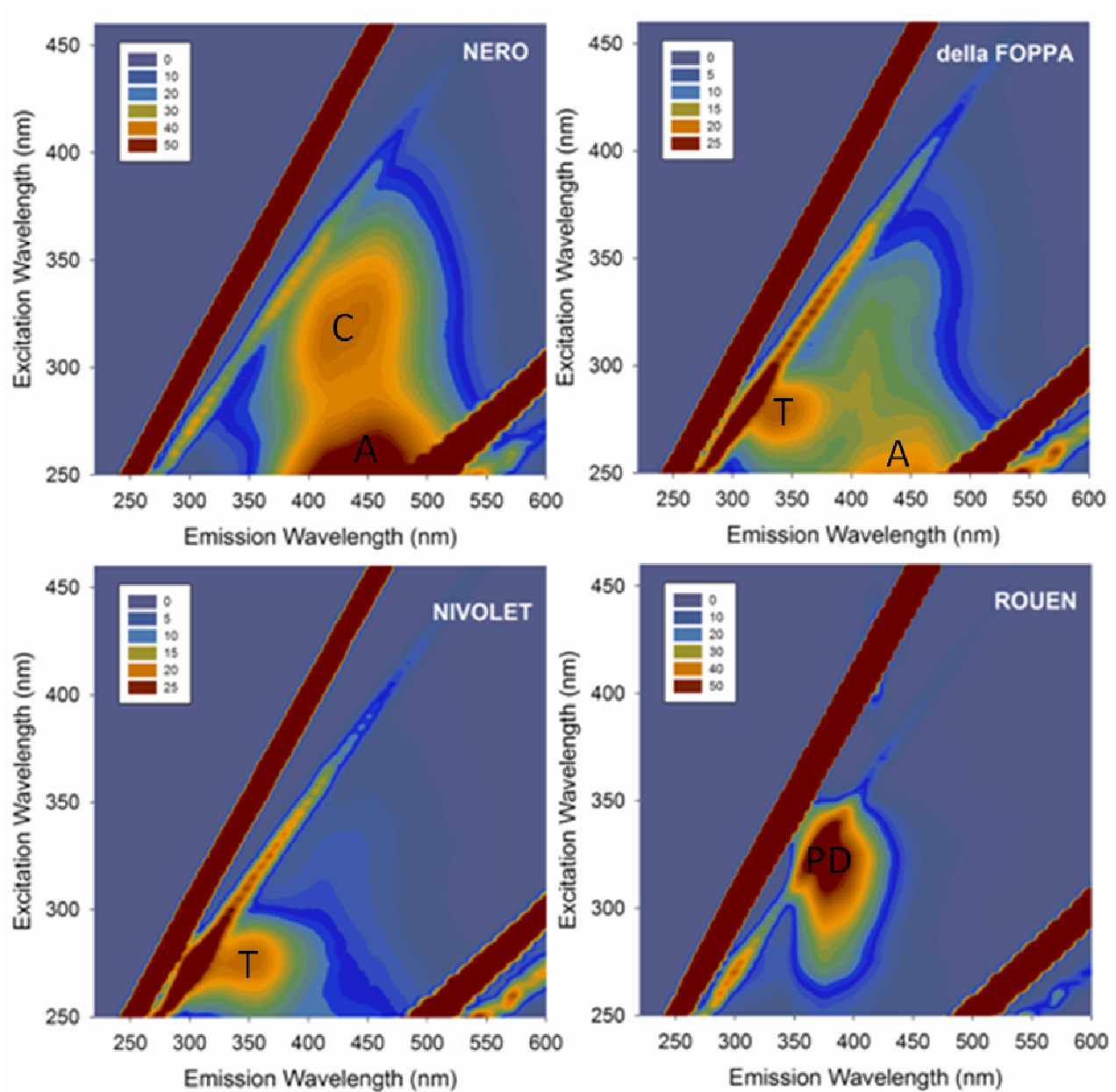




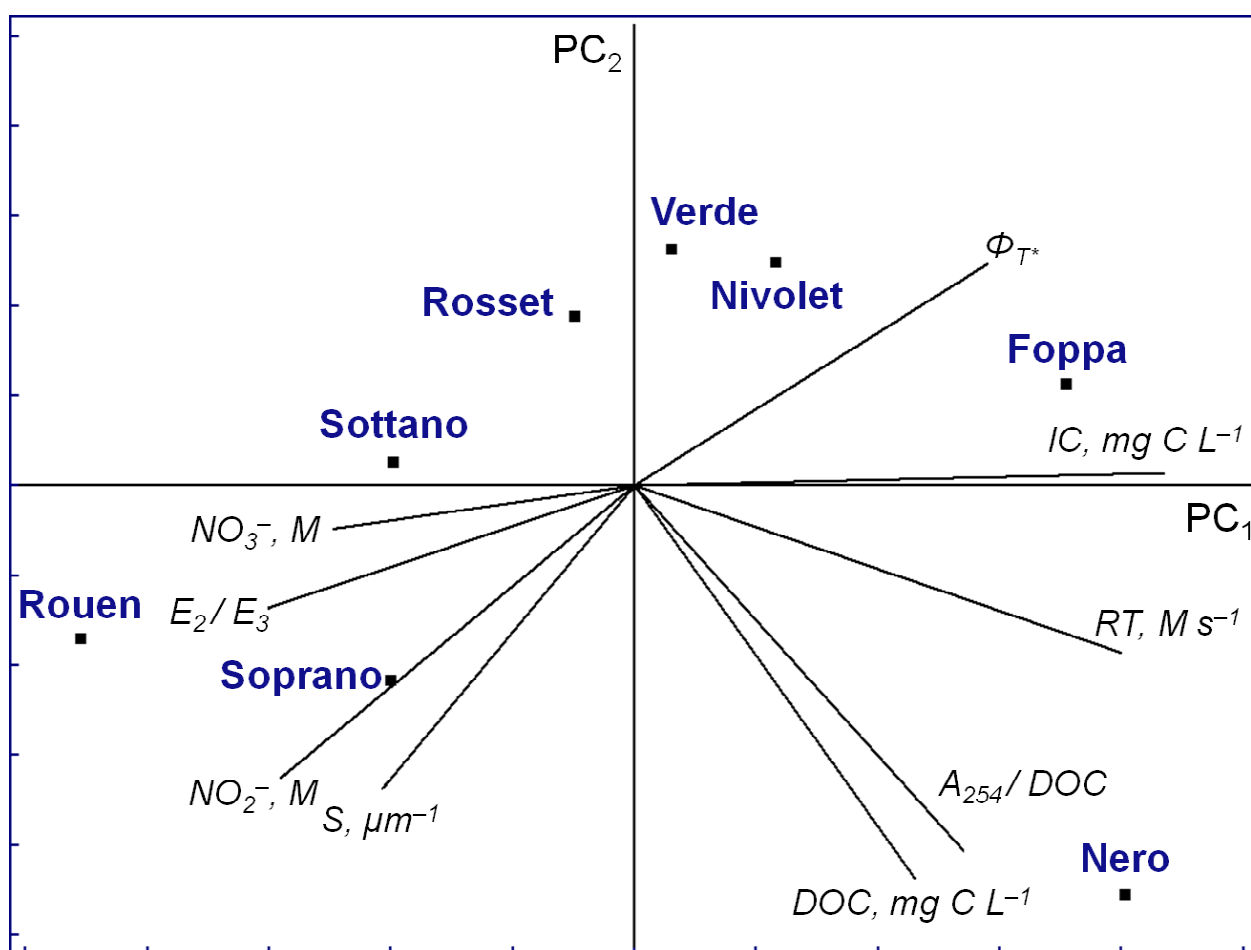
**Figure 1.** Emission spectrum (spectral photon flux density  $p^\circ(\lambda)$ ) of the adopted UVA lamps (Philips TL K05). Absorption spectra of the studied lake water samples ( $A_1(\lambda) = A(\lambda) b^{-1}$ ,  $b = 10$  cm), listed from the most to the least intense.



**Figure 2.** Time evolution of 10  $\mu\text{M}$  TMP, spiked to the lake water samples or to Milli-Q water, upon UVA irradiation. The error bars represent  $\mu \pm \sigma$  of duplicate runs.



**Figure 3.** 3D fluorescence excitation-emission matrix (as contour plot) of Lago Nero, della Foppa, Nivolet and Rouen. Fluorescence intensity is scaled from 0 to 50 intensity units for Lago Nero and Rouen and from 0 to 25 for Lago della Foppa and Nivolet. A, C: humic-like peaks; T: tryptophan-like peak; PD: Phytoplankton Decomposition.



**Figure 4.** Principal Component Analysis (PC1 vs. PC2) of the numerical data reported in Table 1.